J. Physiol. (1940) 98, 361–374 577.174.5:612.464.3

THE INACTIVATION OF ADRENALINE *IN VIVO* IN MAN

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(*Received* 23 *March* 1940)

Experimental work on the sympathetic nervous system has been held up to a considerable extent by our ignorance of the way in which adrenaline is inactivated in the body. In many experiments, for example, in which the effects of adrenaline are measured, the rate of inactivation of adrenaline comes in as an unknown and possibly variable factor affecting the apparent sensitivity of the system to adrenaline. It has frequently been assumed that adrenaline is destroyed in the body by "autoxidation", but the growing evidence that adrenaline may be concerned in the transmission of impulses by the postganglionic sympathetic neurons raises the question of whether there is an enzymic system in the body which inactivates adrenaline in the same way as choline esterase inactivates acetylcholine.

Experiments *in vitro* have shown that adrenaline is readily oxidized by a number of different enzymic systems. These include (*a*) catechol oxidase [Abderhalden & Guggenheim, 1908], (*b*) the cytochrome system [Green & Richter, 1937], (*c*) amine oxidase [Blaschko, Richter & Schlossmann, 1937; Richter, 1937], (*d*) peroxidase and (*e*) *pseudo*phenolases such as the copper-protein complexes [Bhagvat & Richter, 1938]; but it is not yet known to what extent any of these systems are concerned in the inactivation of adrenaline *in vivo*.

Bacq [1938] has supported the view that adrenaline is inactivated by a catechol oxidase, but Bhagvat & Richter [1938] were unable to find an active catechol oxidase in mammalian tissues. Gaddum & Kwiatkowski [1938] concluded that adrenaline is oxidized *in vivo* by the amine oxidase, since the effects of adrenaline are augmented by ephedrine which inhibits this enzyme, and they have put forward a theory of the action of ephedrine which is based on this view; but Richter & Tingey [1939] have

recently shown that the rate of oxidation of adrenaline by the amine oxidase is probably too slow to account for the rate of inactivation observed *in vivo*. It is therefore impossible to draw any conclusions as yet from these *in vitro* experiments as to the way in which adrenaline is inactivated in the tissues *in vivo*.

A new approach to this problem has now been tried by attempting to identify reaction products in the urine after administering adrenaline and other sympathomimetic amines of the adrenaline series. These amines are all toxic substances which can be given only in relatively small amounts, but some of them are easier to investigate in this way than adrenaline as they can be given in much larger doses.

The inactivation of adrenaline by the amine oxidase might be expected to lead to the formation of protocatechuic acid, $(OH)_2C_6H_3COOH$, which would be excreted in conjugated form in the urine. Weinstein & Manning [1937] have reported the excretion of a substance similar to protocatechuic acid, after giving large doses of adrenaline, in the rabbit. Inactivation by catechol oxidase, the cytochrome system or *pseudo*-phenolases forms adrenochrome which is an indol derivative and might be expected to appear in the urine as a conjugated indole derivative.

The experiments with corbasil and other amines were done to obtain further evidence as to the activity *in vivo* of the different inactivating systems.

Corbasil

dl-Corbasil, $(OH)_2C_6H_3.CHOH.CH(NH_2)CH_3$, which is derived from ephedrine, was chosen for examination in the first place since it is not oxidized by the amine oxidase and inactivation by this enzyme could therefore be excluded. It has previously been shown that amines such as ephedrine, which are not oxidized by the amine oxidase, are generally excreted in the urine unchanged [Richter, 1938], and it was expected that corbasil would also be excreted unchanged unless inactivated by another system.

Preliminary experiments showed that added corbasil could be separated from urine by adsorption on aluminium hydroxide at *p*H 9 and elution with phosphoric acid. It could then be estimated by the iodine method commonly used for estimating adrenaline.

Corbasil is known to be active when taken by mouth [Hartung, Munch, Miller & Crossley, 1931]. Doses up to 50 mg. were taken by the author (76 kg.) by mouth, but no free corbasil could be found in the urine although the methods used were sufficiently sensitive to detect an excretion of 1 mg./hr. or 2%/hr. of the amount taken.

In order to test for the presence of conjugated derivatives the urine was hydrolysed by heating for 1 hr. with 10% sulphuric acid. It was then found to contain a phenolic compound which was readily separated from the urine by adsorption on aluminium hydroxide and identified as corbasil by (*a*) the green colour reaction with ferric chloride, (*b*) the red colour fading to yellow given with aqueous iodine in potassium iodide, and (*c*) the characteristic violet colour, extractable by butyl alcohol, given by treating at *p*H 5·2 with alcoholic iodine followed by sodium thiosulphate.

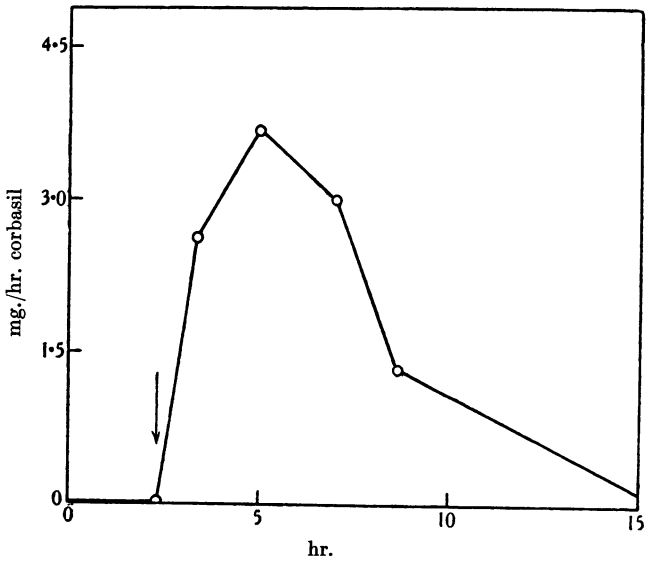

Fig. 1. Rate of elimination of conjugated corbasil derivative after taking 50 mg. at time marked by arrow.

The last colour reaction is due to the formation of an iodoadrenochrome derivative [Richter & Blaschko, 1937] and is very specific for amines of the adrenaline series. The corbasil obtained from the hydrolysed urine gave a brilliant blue-violet colour identical in shade with that given by pure corbasil.

Rate of elimination. Urine was collected every 3–4 hr. after taking corbasil (50 mg. base with 0·2 g. glycine and 2 ml. *N*/5 hydrochloric acid to reduce oxidation in the intestine). Conjugated corbasil was estimated in the urine by the method described below for adrenaline. The rate of excretion of conjugated corbasil was maximal from 2 to 6 hr. after administration (Fig. 1) and was still considerable after 9 hr. The total

amount of corbasil found in the hydrolysed urine up to 9 hr. after administration was 24 mg. or 48% of the amount taken, but the elimination was probably not complete in that time.

Epinine

Epinine, $(OH)_2C_6H_3.CH_2CH_2NHCH_3$, is oxidized much more rapidly than adrenaline by the amine oxidase, and might therefore be expected to be oxidized by this enzyme *in vivo* and eliminated as protocatechuic acid, unless conjugation and elimination in the same way as corbasil is a more rapid process. Urine was collected before and after taking 50 mg. epinine by mouth (61 mg. hydrochloride with 0·2 g. glycine and 1 ml. 5% acetic acid in 50 ml. water). No free epinine was found in the urine on testing by the colour reaction with iodine.

Test for protocatechuic acid. The specimens of urine (40 ml.) were heated with 3 ml. conc. H_2SO_4 on the boiling water-bath for 30 min., cooled and extracted with 150 ml. ether. The ether was now extracted with 20 ml. 5% sodium bicarbonate, the extract shaken with an excess of solid sulphanilic acid and treated with 0·1 ml. $N/10$ iodine solution in 5% potassium iodide. Under these conditions protocatechuic acid gives a red colour visible at concentrations down to 0·02 mg./ml. The tests on the urine were all negative.

Test for conjugated epinine. The urine was treated with an excess of 25% lead acetate and filtered. The filtrate was brought to *p*H 8 with 5% ammonia solution (cresol red), allowed to stand for 1 hr. and the precipitate collected by decanting the clear solution and centrifuging the residue. The precipitate was decomposed with a slight excess of N sulphuric acid (acid to congo red) and the lead sulphate centrifuged off. The resulting solution, after hydrolysis under the conditions described, gave the characteristic reactions of epinine with ferric chloride and aqueous and alcoholic iodine.

The colour given by epinine with iodine is an orange red which is not suitable for quantitative estimation in the presence of the urinary pigments, but a rough colorimetric estimation indicated that the amount of conjugated epinine in the urine was between 30–60% of the amount taken.

The green colour given by epinine with ferric chloride depends on the presence of the catechol group. Since this reaction was positive after, but negative before, hydrolysis, the conjugated derivative could not contain a free catechol group; this means that conjugation must occur at one of the phenolic hydroxyl groups.

d-ADRENALINE

d-Adrenaline, $(OH)_2C_6H_3.CHOH.CH_2NHCH_3$, differs from the natural *l*-adrenaline only in the spatial configuration of the CHOH group; it is oxidized by the amine oxidase and cytochrome system but not quite as rapidly as the naturally occurring isomer.

Urine was collected before and after taking 55 mg. *d*-adrenaline (100 mg. *d*-adrenaline ditartrate with 0·2 g. glycine and 1 ml. 2% acetic

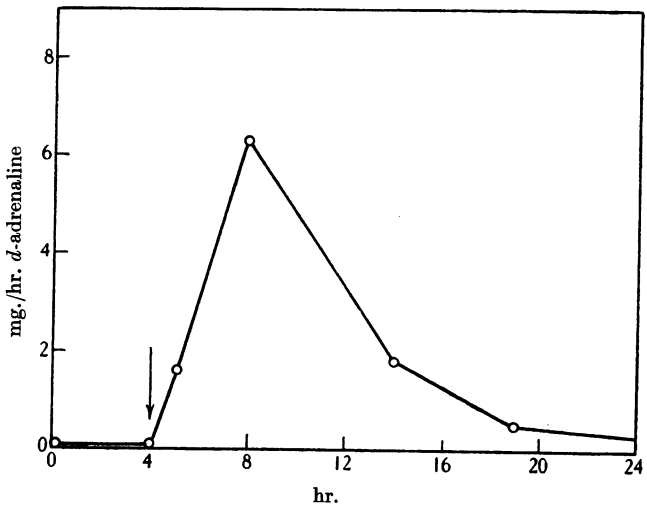

Fig. 2. Rate of elimination of conjugated *d*-adrenaline in urine after taking 55 mg. at time marked by arrow.

acid in 50 ml. by mouth). No free or combined protocatechuic acid and no free *d*-adrenaline was found in the urine, but after hydrolysis with acid *d*-adrenaline could be separated by adsorption on aluminium hydroxide and elution with phosphoric acid; it gave the green ferric chloride reaction and characteristic violet colour of iodoadrenochrome with alcoholic iodine followed by sodium thiosulphate at *p*H 5·2.

Rate of elimination. Conjugated *d*-adrenaline was estimated in the urine by the method given below for *l*-adrenaline. The rate of elimination was maximal some 4 hr. after administration (Fig. 2). The amount of *d*-adrenaline found in the urine after hydrolysis was 39·2 mg. in 21 hr. after administration or 71% of the amount taken.

l-Adrenaline

Activity of adrenaline when taken by mouth. It is commonly stated in the literature that adrenaline is inactive when given by mouth [Gunn, 1939; Martindale, 1936; Tuohy & Essex, 1937], though it has been shown repeatedly that the effects of adrenaline are obtained when it is given by mouth in animals [Menninger, 1927; Dorlencourt, Trias & Paychère, 1922; Giragossintz & Mackler, 1929]. It is to be expected that adrenaline

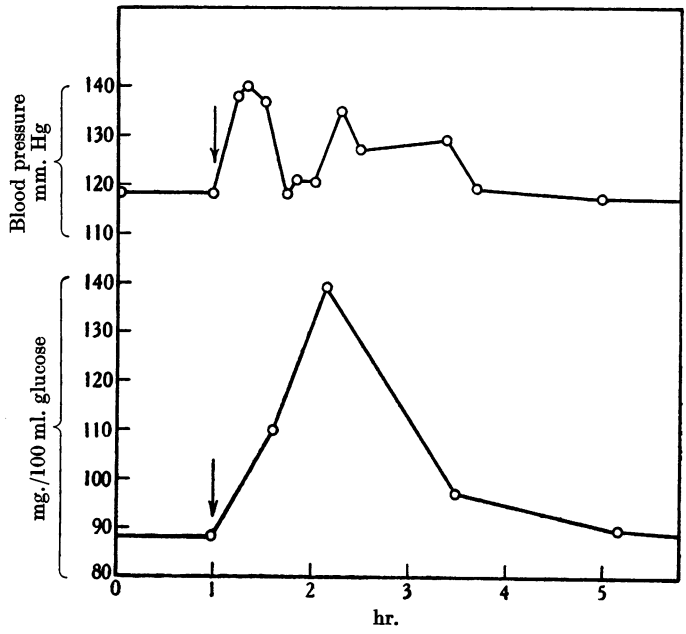

Fig. 3. Systolic blood pressure and blood sugar (by Hagedorn & Jensen method) after taking 15 mg. *l*-adrenaline by mouth at time marked by arrows.

will be partially oxidized at the alkaline *p*H of the intestine unless precautions are taken to prevent this, but the most probable reason for the apparent discrepancy in the literature is that when adrenaline is given by mouth it is considerably diluted and in part inactivated during the passage through the liver before it reaches the general circulation; much larger doses are therefore required to produce a given effect than when it is given subcutaneously or intravenously. In agreement with this view it has been shown that the effects produced by adrenaline are much less when it is injected into the portal vein than when injected into the peripheral veins [Markowitz & Mann, 1929].

The normal dose of adrenaline for subcutaneous injection in man is about 1 mg. Doses of 15 mg. adrenaline taken with 0·1 g. glycine and 10 ml. 1% acetic acid in 50 ml. water by the author by mouth 4 hr. after a meal caused marked blanching of the skin, sweating on the face, diarrhoea, a feeling of abdominal discomfort, a rise of 20 mm. in the systolic blood pressure and a rise of 51 mg./100 ml. in the blood sugar (Fig. 3). A dose of 30 mg. adrenaline taken with 0·1 g. glycine and 10 ml. 1% acetic acid in 50 ml. water caused, in addition to these effects, marked glycosuria and severe abdominal pains which persisted for several hours. It was therefore evident that adrenaline was active when taken by mouth when precautions were taken to prevent oxidation in the intestine.

Elimination. Analogy with corbasil, epinine and *d*-adrenaline suggested that *l*-adrenaline might also be eliminated by conjugation, though in view of its physiological significance it was possible that there might be an entirely different system specially designed for the inactivation of *l*-adrenaline. Examination of the urine after taking 10–30 mg. adrenaline failed to show the presence of any free adrenaline, but after hydrolysing with acid the presence of adrenaline was shown by every test that was applied. The isolation of small amounts of adrenaline from relatively large amounts of urine presents a technical problem that has not yet been solved, but fortunately adrenaline gives a number of very specific colour reactions which enable it to be identified with a high degree of certainty without actual isolation. The tests applied were best shown after partial purification of the conjugated adrenaline derivative by lead acetate precipitation and extraction with alcohol as described below: the adrenaline derivative was then hydrolysed by heating for 30 min. with 10% sulphuric acid. The tests include (*a*) the green colour reaction with ferric chloride, due to the catechol nucleus, (*b*) the red colour fading to yellow with aqueous iodine in KI followed by sodium thiosulphate, due to the formation of adrenochrome, (*c*) the orange colour given on treating adrenochrome with hydroxylamine, due to the formation of adrenochrome oxime [Green & Richter, 1937], (*d*) the violet colour on warming adrenochrome with Ehrlich's reagent (*p*-dimethylaminobenzaldehyde), due to the indole structure, (*e*) the violet colour with alcoholic iodine at *p*H 5·2 followed by sodium thiosulphate, due to iodoadrenochrome [Richter & Blaschko, 1937], (*f*) the reduction of arsenomolybdic acid and specific test with NaOH described by Shaw [1938], and (*g*) the inhibition of isolated strips of rabbit intestine. Tests for free or conjugated protocatechuic acid or for an increased elimination of indole derivatives, using Ehrlich's reagent, were negative. It was concluded that adrenaline was

eliminated in the same way as the other dihydroxyphenylethylamine derivatives, by conjugation.

Hydrolysis of conjugated adrenaline. In order to determine the optimum conditions for hydrolysis flasks containing 40 ml. urine collected after taking 15 mg. adrenaline were heated with 3 ml. conc. H_2SO_4 for varying periods on a boiling water-bath and the amount of free adrenaline formed was estimated by the method described below. The optimum time of hydrolysis was about 30 min. and there was a marked falling off in the amount of adrenaline found with longer periods (Fig. 4); this indicated that the hydrolysis was not entirely quantitative, but the amount of adrenaline lost in 30 min. was probably not large.

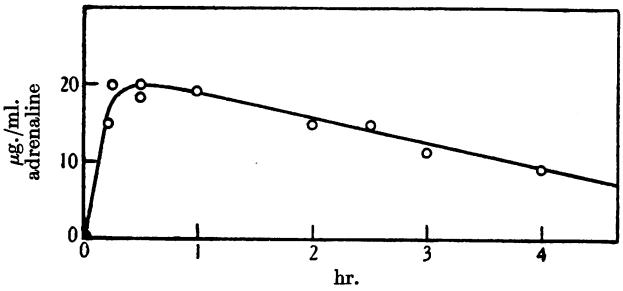

Fig. 4. *l*-Adrenaline estimated in urine containing conjugated derivative after hydrolysing for different times.

Estimation of conjugated adrenaline. Attempts to estimate adrenaline in the urine by the arsenomolybdate method of Shaw [1938] were only partially successful since it gave a high blank value which had to be subtracted from the figures obtained with adrenaline. The following method, which is similar to that described by Richter & Tingey [1940], was found to be more satisfactory and was generally used:

The urine was hydrolysed by heating 40 ml. with 3 ml. conc. H_2SO_4 in a 50 ml. measuring flask for 30 min. on a boiling water-bath. To 15 ml. of the hydrolysed urine was added 0·5 ml. 1·5% glycine, 0·2 ml. saturated sodium acetate and sufficient thymol blue to give a clear red colour. The mixture was cooled by shaking in a wide test-tube in a basin of cold water and brought to *p*H 4–6 by dropping in 40% sodium hydroxide, care being taken to avoid an excess. A precipitate which formed was filtered off at once and the residue on the filter-paper washed through with 10 ml. water. An aluminium hydroxide suspension (2 ml.) was now added, the mixture brought to a grey-blue colour with 8% sodium hydroxide, centrifuged and the supernatant solution discarded. (The aluminium hydroxide suspension was prepared by adding 10 g. sodium hydroxide in 40 ml. water to a solution prepared by dissolving 50 g. potassium alum in 400 ml. hot water and cooling to about 20°: the precipitate was filtered, washed twice with water and suspended in 200 ml. water.) The precipitate containing adsorbed adrenaline was shaken with 1 ml. 25% sodium dihydrogen phosphate and 6 ml. water. The solution obtained on centrifuging again was decanted into a

clean test-tube and treated with 0·4 ml. 5 *M* sodium acetate buffer *p*H 5·2 and 0·5 ml. *N*/10 iodine in 5% KI. After standing for exactly 5 min. 0·5 ml. sodium thiosulphate was added. The red colour was compared at once with the colour in a set of standard tubes prepared by adding known amounts of adrenaline to hydrolysed urine and treating in the same way. No great accuracy can be claimed for this method as pigments derived from the urine frequently made the colorimetric comparison difficult; but with an adrenaline excretion of 1 mg./hr. or more the figures were probably accurate to within 10%.

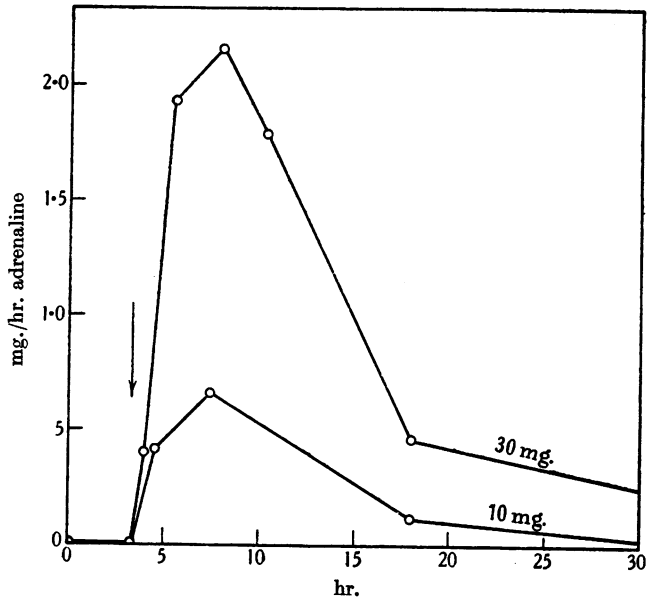

Fig. 5. Rate of elimination of adrenaline derivative in the urine after taking (*a*) 10 mg. and (*b*) 30 mg. *l*-adrenaline at the time marked by arrow.

Rate of elimination. Urine was collected and conjugated adrenaline estimated before and after taking 10–30 mg. adrenaline. With 30 mg. adrenaline the rate of elimination was maximal in about 5 hr. and then slowly decreased in the course of 24 hr. (Fig. 5).

In several experiments the total amount of adrenaline found in the hydrolysed urine was estimated. The results, with those obtained for other amines of this series, are summarized in Table I.

The figures given for the percentage recovery in the urine must be regarded as tentative since (*a*) urinary pigments interfered to some extent in the estimation, (*b*) there was evidence of some loss during the hydrolysis (Fig. 4), and (*c*) the rate of elimination decreased asymptotically so that the elimination was not complete in the periods during which the urine was collected.

TABLE I. Recovery of amines in the urine

Exp.		Dose mg. base/ 76 kg.	Time urine was collected hr.	Amount found in urine mg.	Percentage recovery
1	Corbasil	50	9	24	48
2	Epinine	50	15	15–30	30–60
3	*d*-Adrenaline	55	21	39·2	71
4	*l*-Adrenaline	10	7	3–6	30–60
5	,,	10	20	5·5	55
6	,,	15	9	10·5	70
7	,,	30	20	20·7	69

(Estimations by the iodine method except in Exp. 5 in which the arsenomolybdate method was used.)

The concentration of conjugated adrenaline in the urine rose to a value corresponding to 33 μg. adrenaline/ml. in the urine obtained 5 hr. after taking 30 mg. adrenaline. Taking the blood volume as 5 l., the concentration in the blood could not be higher than 6 μg./ml. so that the kidneys must be able to concentrate the derivative to a considerable extent.

Purification of conjugated adrenaline derivative. Attempts to isolate the adrenaline derivative from the urine have not hitherto succeeded, but methods have been worked out which enabled a partial purification to be effected. In this work various methods of fractional precipitation, etc., were tried and the behaviour of the adrenaline derivative was followed by hydrolysing the fractions and estimating adrenaline as described.

The adrenaline derivative was precipitated by lead acetate at alkaline but not at neutral *p*H, and by making use of this it could be separated from a large number of other substances present in the urine. An excess of 25 % lead acetate solution was added to the urine, the bulky precipitate filtered off and the filtrate adjusted to *p*H 9 with ammonia. On standing, a fine precipitate separated. This was collected, washed and decomposed by adding dilute sulphuric acid (strongly acid to congo red). The adrenaline derivative was found in the clear solution obtained on centrifuging.

A further considerable purification was obtained by evaporating the aqueous solution nearly to dryness and extracting with a small volume of 95 % alcohol. This dissolved most of the adrenaline derivative and left a gummy residue of impurities. On fractionally precipitating the alcoholic solution with barium hydroxide the adrenaline derivative was present mainly in the later fractions. When the barium compound was decomposed with sulphuric acid and the resulting solution, after neutralizing with sodium hydroxide, was slowly evaporated in a desiccator, various

sodium salts crystallized out and were separated. The adrenaline derivative was then left as a hygroscopic amorphous mass. Many attempts to crystallize a potassium or a barium salt were unsuccessful.

The conjugated adrenaline derivative differed from adrenaline in that it was not adsorbed on aluminium hydroxide at *p*H 9 and this could be used for separating them. It was very soluble in water, soluble in 90% alcohol, less soluble in absolute alcohol, slightly soluble in ether, insoluble in chloroform. It was decomposed by heating for 30 min. with dilute sodium carbonate.

Chemical constitution of conjugated derivative. The conjugated adrenaline did not give the green colour characteristic of the catechol grouping with ferric chloride, though this reaction was readily obtained after hydrolysis. This indicated that conjugation had occurred at one of the phenolic hydroxyl groups.

Analogy with the elimination of other phenols made it appear likely that adrenaline may be eliminated either as a sulphate ester or as a glycuronide. Partially purified specimens of the adrenaline derivative gave negative glycuronide tests with naphthoresorcinol and showed less than 20% hydrolysis after incubation for 5 hr. at 37° with emulsin; tests for a sulphate ester were positive. No final conclusions can be drawn until the conjugated adrenaline derivative has been isolated in the pure state, but these preliminary experiments show that it has the properties of an ester in that it is easily hydrolysed by acids and suggest that it may be adrenaline-3 or 4-*o*-hydrogen sulphate, which would normally exist as a zwitterion, $\overline{SO_3}O(OH)C_6H_3.CHOH.CH_2.\overset{+}{N}HCH_3$.

Elimination of adrenaline in the rabbit. A large rabbit (2·5 kg.) was given 20 mg. adrenaline with 0·1 g. glycine and 1 ml. 1% acetic acid in 20 ml. water. No free adrenaline was found in the urine, but the presence of conjugated adrenaline was indicated by positive tests for adrenaline after hydrolysis with acid. Weinstein & Manning [1937] reported the excretion of protocatechuic acid after administering adrenaline; but they hydrolysed the urine by heating with alkali. Since protocatechuic acid is formed from adrenaline on heating with alkali, it may have come from adrenaline in the urine. The excretion of protocatechuic acid in the rabbit would therefore appear to require reinvestigation.

Discussion

The experiments described give evidence that adrenaline administered in doses of 0·13–0·4 mg./kg. in man is mainly eliminated in the urine in the form of a derivative which has the properties of an adrenaline ester in

which esterification has occurred at one of the phenolic hydroxyl groups. This ester has not yet been obtained pure, but preliminary experiments suggest that it may be the sulphate ester and the inactivating system the "sulphosynthase" or system responsible for the conjugation of phenols with sulphate in the body.

The observation that adrenaline is eliminated by conjugation instead of being oxidized by the amine oxidase in the same way as phenylethylamine and most other amines [Richter, 1938], indicates that conjugation is more rapid than oxidation by the amine oxidase and suggests that it is probably the main physiological method by which adrenaline is inactivated in the body. The detoxication of phenols by conjugation is known to occur mainly in the liver [Pelkan & Whipple, 1922] and this agrees with the physiological evidence that the liver is also particularly effective in removing adrenaline from the circulation [Elliott, 1905; Trendelenburg, 1929].

The simplest interpretation of the present observations is that there is no special system in the body for the inactivation of adrenaline, but that it is detoxicated by conjugation in the same way as other toxic phenols. On the other hand, the work of Quick [1932] challenges the view that the "sulphosynthase" and "glycuronidosynthase" are primarily concerned in detoxication and indicates that these systems may have some more important physiological function. It is suggestive that under normal conditions the conjugation of the simple phenols by these systems is generally incomplete and a considerable proportion of the phenol may be eliminated unchanged [Novello, Wolf & Sherwin, 1925], while with adrenaline the conjugation was remarkably complete and no unchanged adrenaline was ever found in the urine. This suggests that the inactivating system may be specially adapted for inactivating adrenaline, which is probably more toxic and of greater physiological significance than the other phenols normally eliminated in this way.

It should be possible to test experimentally the view that adrenaline is normally inactivated by conjugation by administering other phenols, which should inhibit the inactivation by competing with adrenaline for the inactivating system and should so increase and prolong the effects of adrenaline. This experiment has already been carried out. Many years ago Barger & Dale [1910] observed the effect of catechol in raising the blood pressure, and more recently Bacq [1936] has shown with a series of polyphenols that they augment and prolong the effects of adrenaline and of stimulating the sympathetic nerves *in vivo*.

Bacq concluded that the polyphenols act by inhibiting the autoxida-

tion of adrenaline since phenols have been shown to act as autoxidation inhibitors *in vitro*. This interpretation is very improbable since the tissues already contain autoxidation inhibitors which are far more powerful than the relatively small amounts of phenols administered: there is also no evidence that adrenaline is oxidized *in vivo*. The present work suggests that adrenaline is mainly inactivated by conjugation and that the polyphenols act in augmenting and prolonging the effects of adrenaline by inhibiting this process. In so far as it is possible to draw an analogy between the adrenergic and cholinergic mechanisms, the parts played by eserine and choline esterase would appear to be represented in the adrenergic system by the polyphenols and "sulphosynthase".

Summary

1. Corbasil, epinine and *d*- and *l*-adrenaline administered in doses of 0·13–0·66 mg./kg. by mouth in man are mainly eliminated in the form of conjugated derivatives in the urine.

2. Conjugation occurs on one of the phenolic hydroxyl groups and the derivatives formed are probably the sulphate esters.

3. The rates of elimination of conjugated corbasil and *d*- and *l*-adrenaline have been measured.

4. Up to 70% of the amounts of adrenaline administered were found in the urine after hydrolysis.

5. Adrenaline is active when given by mouth if precautions are taken to prevent oxidation in the gut.

6. Epinine and *d*- and *l*-adrenaline are not eliminated to any measurable extent as protocatechuic acid.

7. It is concluded that conjugation is the main physiological process by which adrenaline is inactivated in the body and that the inactivation of adrenaline in this way may be intimately related to the observed activity of adrenaline and the functioning of the sympathetic nervous system *in vivo*.

The author wishes to thank Prof. S. Nevin for his interest and the Rockefeller Foundation for supporting this investigation.

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